

**What is claimed is:**

1. A method for quantifying the amount of components in a sample of material selected from unfractionated heparins and fractionated heparins, comprising:
  - (a) depolymerizing said sample by an enzymatic method; and
  - (b) detecting the quantity of the components in the depolymerized sample of step (a) by high-performance liquid chromatography.
2. The method as claimed in claim 1, wherein the enzymatic method is carried out using at least one heparinase.
3. The method as claimed in claim 1, wherein the enzymatic method is carried out using a mixture of heparinase 1 (EC 4.2.2.7.), heparinase 2 (heparin lyase II), and heparinase 3 (EC 4.2.2.8.).
4. The method as claimed in claim 1, wherein the fractionated heparin is enoxaparin sodium.
5. The method as claimed in claim 1, wherein the high performance liquid chromatography used in step (b) is anion-exchange chromatography.
6. The method as claimed in claim 1, wherein the high performance liquid chromatography used in step (b) is strong anion exchange chromatography (SAX).
7. The method as claimed in claim 6, wherein the strong anion exchange chromatography is carried out using a Spherisorb® SAX column.
8. The method as defined in claim 1, wherein the high-performance liquid chromatography is carried out in a mobile phase which is transparent to UV light with wavelengths from about 200 nm to about 400 nm.

9. The method as claimed in claim 1, wherein the high-performance liquid chromatography is carried out in a mobile phase which comprises at least one salt chosen from sodium perchlorate, methanesulfonate salts, and phosphate salts.

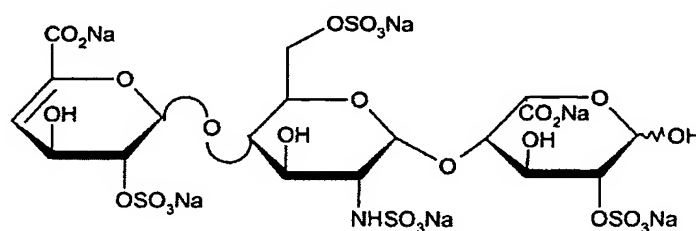
10. The method as claimed in claim 1, wherein the high-performance liquid chromatography is carried out in a mobile phase which comprises sodium perchlorate salts.

11. The method as claimed in claim 6, wherein the strong anion exchange chromatography is carried out at a pH of about 2.0 to about 6.5.

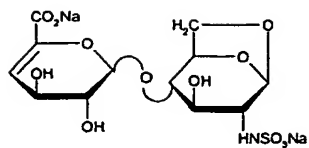
12. The method as claimed in claim 6, wherein the strong anion exchange chromatography is carried out at a pH of about 3.

13. The method as claimed in claim 1, wherein the mobile phase comprises a sodium perchlorate solution that is maintained at about pH 3.0.

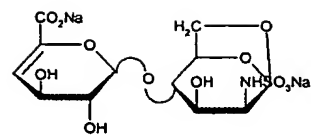
14. The method as claimed in claim 1, wherein the depolymerized sample comprises at least one oligosaccharide chain selected from any one of the following



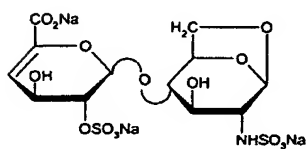
trisaccharide 1



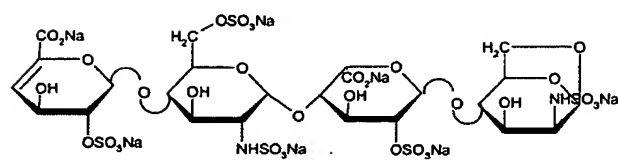
disaccharide 1



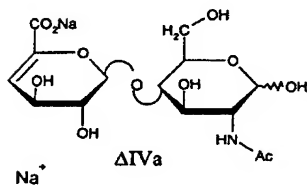
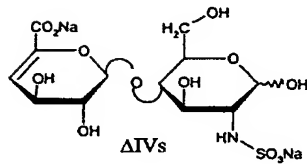
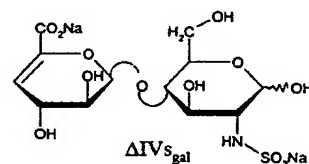
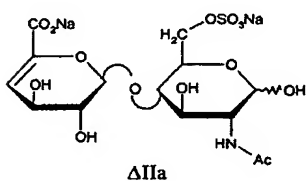
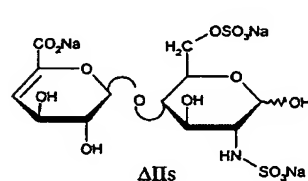
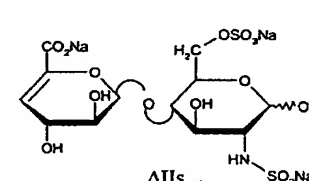
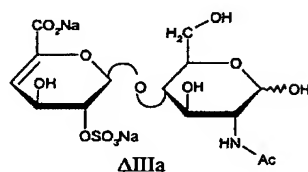
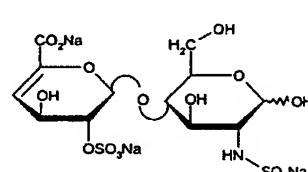
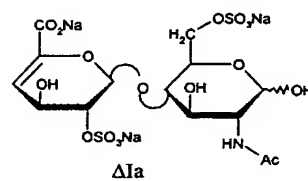
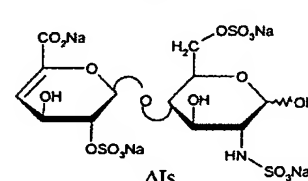
disaccharide 2

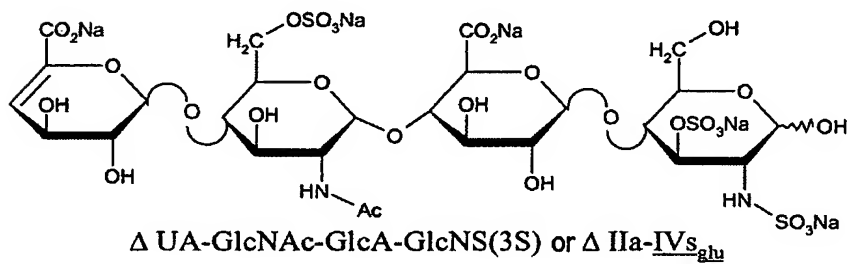
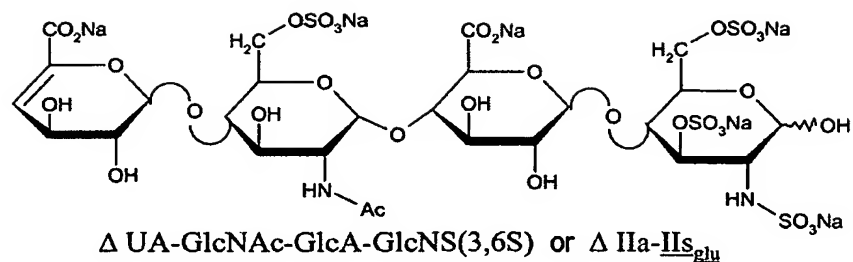


disaccharide 3

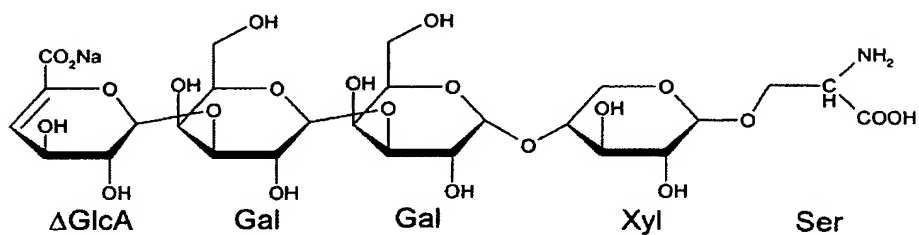


tetrasaccharide 1

 $\Delta IVa$  $\Delta IVs$  $\Delta IVsgal$  $\Delta IIa$  $\Delta II_s$  $\Delta IIsgal$  $\Delta IIIa$  $\Delta III_s$  $\Delta Ia$  $\Delta I_s$

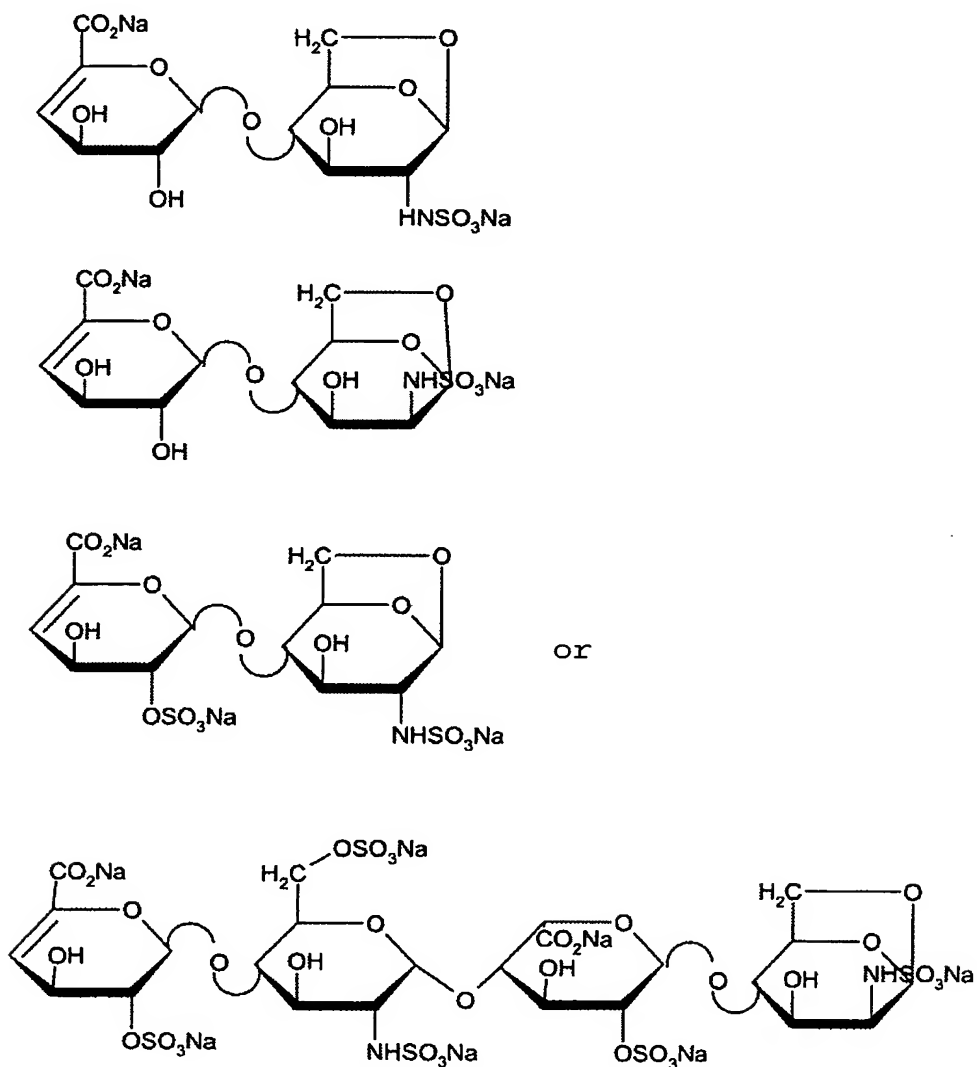


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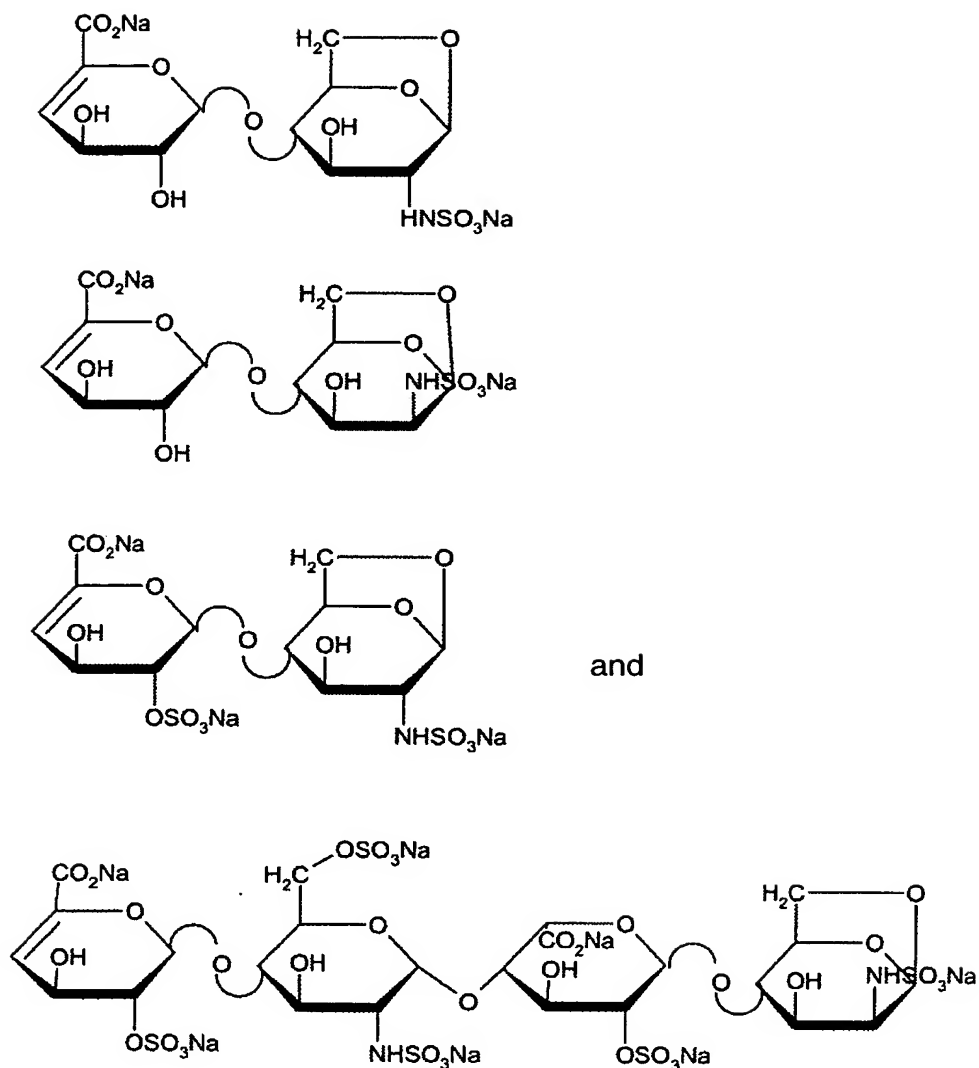


15. The method as claimed in claim 1 wherein the depolymerized sample comprises at least one oligosaccharide chain whose end is modified with a 1,6-anhydro bond.

16. The method as claimed in claim 15, wherein the at least one oligosaccharide chain is chosen from any of the following



17. The method as claimed in claim 4, wherein the depolymerized sample comprises at least one 1,6-anhydro residue chosen from any of the following:



18. The method as claimed in claim 17, wherein the at least one 1,6-anhydro residue ranges from 15% to 25% of the weight average molecular weight of the sample.

19. The method as claimed in claim 1, wherein the components detected in the depolymerized sample of step (b) are acetylated sugars.

20. The method as claimed in claim 19, wherein the acetylated sugars are selectively detected by subtracting an absorbance measured at a wavelength at which both acetylated and nonacetylated sugars absorb from an absorbance measured at a wavelength at which acetylated but not nonacetylated sugar absorbs.

21. The method as claimed in claim 19, wherein the acetylated sugars detected are selected from acetylated oligosaccharides  $\Delta IVa$ ,  $\Delta IIa$ ,  $\Delta IIIa$ ,  $\Delta Ia$ ,  $\Delta IIa-IVs_{glu}$ , and  $\Delta IIa-IIIs_{glu}$ .

22. A method for quantifying the amount of 1,6-anhydro residues in a sample of enoxaparin sodium, comprising:

- (a) depolymerizing said sample using a mixture of heparinase 1 (EC 4.2.2.7.), heparinase 2 (heparin lyase II), and heparinase 3 (EC 4.2.2.8.); and
- (b) detecting the quantity of the 1,6-anhydro residues in the depolymerized sample of step (a) by high-performance liquid chromatography.

23. A method as claimed in claim 22, wherein the quantity of the 1,6-anhydro residues range from 15% to 25% of the mean oligosaccharide molecular weight of the sample.

24. A method for quantifying the amount of components in a sample of material chosen from unfractionated heparins and fractionated heparins, comprising:

- (a) depolymerizing said sample by an enzymatic method; and
- (b) reducing the depolymerized sample of step (a);
- (c) detecting the quantity of the components in the reduced sample of (b) by high-performance liquid chromatography.

25. The method as claimed in claim 24, wherein the enzymatic method is carried out using at least one heparinase.

26. The method as claimed in claim 24, wherein enzymatic method is carried out using a mixture of heparinase 1 (EC 4.2.2.7.), heparinase 2 (heparin lyase II), and heparinase 3 (EC 4.2.2.8.).

27. The method as claimed in claim 24, wherein reducing the depolymerized sample of step (a) is carried out by exposure to a reducing agent.

28. The method as claimed in claim 27, wherein the reducing agent is  $\text{NaBH}_4$  or an alkali metal salt of the borohydride anion.

29. The method as claimed in claim 24, wherein the fractionated heparin is enoxaparin sodium.

30. The method as claimed in claim 27, wherein the reducing reduces the reducing ends of enoxaparin sodium which are not in the 1,6-anhydro form.

31. The method as claimed in claim 24, wherein the high performance liquid chromatography used in step (c) is anion-exchange chromatography.

32. The method as claimed in claim 24, wherein the high performance liquid chromatography used in step (c) is strong anion exchange chromatography (SAX).

33. The method as claimed in claim 32, wherein the strong anion exchange chromatography is carried out using a Spherisorb® SAX column.

34. The method as defined in claim 24, wherein the the high-performance liquid chromatography is carried out in a mobile phase which is transparent to UV light with wavelengths from about 200 nm to about 400 nm.

35. The method as claimed in claim 24, wherein the high-performance liquid chromatography is carried out in a mobile phase which comprises at least one salt chosen from sodium perchlorate, methanesulfonate salts, and phosphate salts.

36. The method as claimed in claim 24, wherein the high-performance liquid chromatography is carried out in a mobile phase which comprises sodium perchlorate salts.

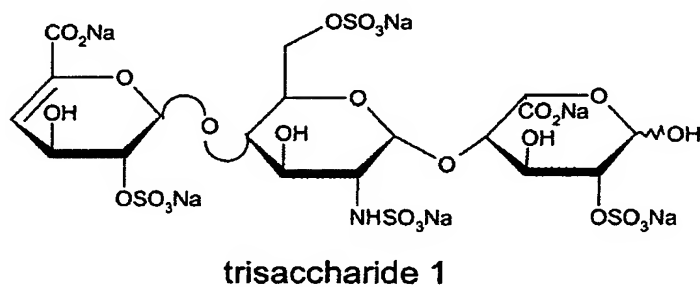
37. The method as claimed in claim 32, wherein the strong anion exchange chromatography is carried out at a pH of about 2.0 to about 6.5.

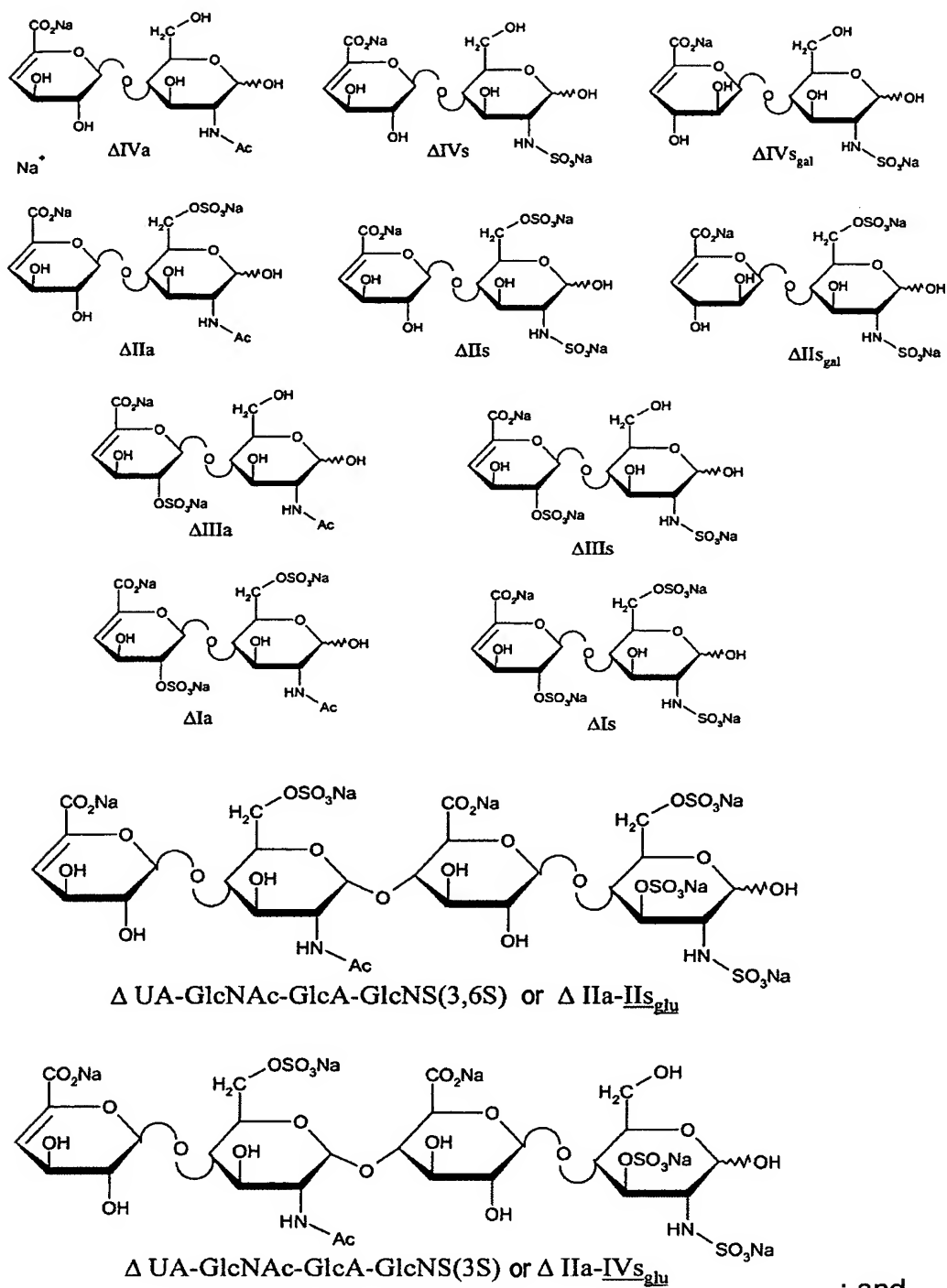


38. The method as claimed in claim 32, wherein the strong anion exchange chromatography is carried out at a pH of about 3.

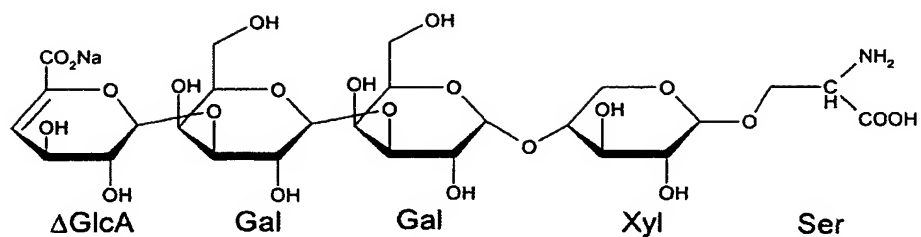
39. The method as claimed in claim 24, wherein the high performance liquid chromatography utilizes a mobile phase comprising a sodium perchlorate solution that is maintained at about pH 3.0.

40. The method as claimed in claim 28, wherein the depolymerized sample comprises at least one oligosaccharide chain selected from any of the following:





; and

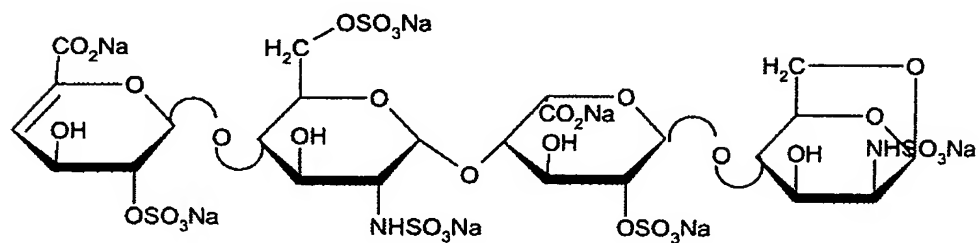
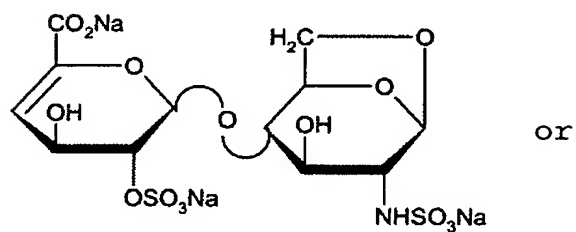
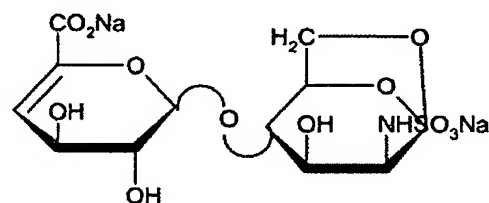
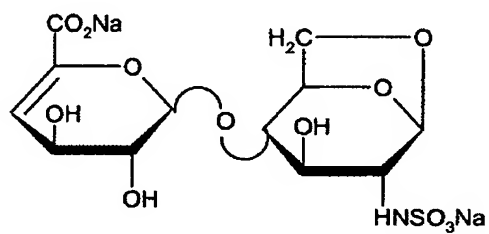


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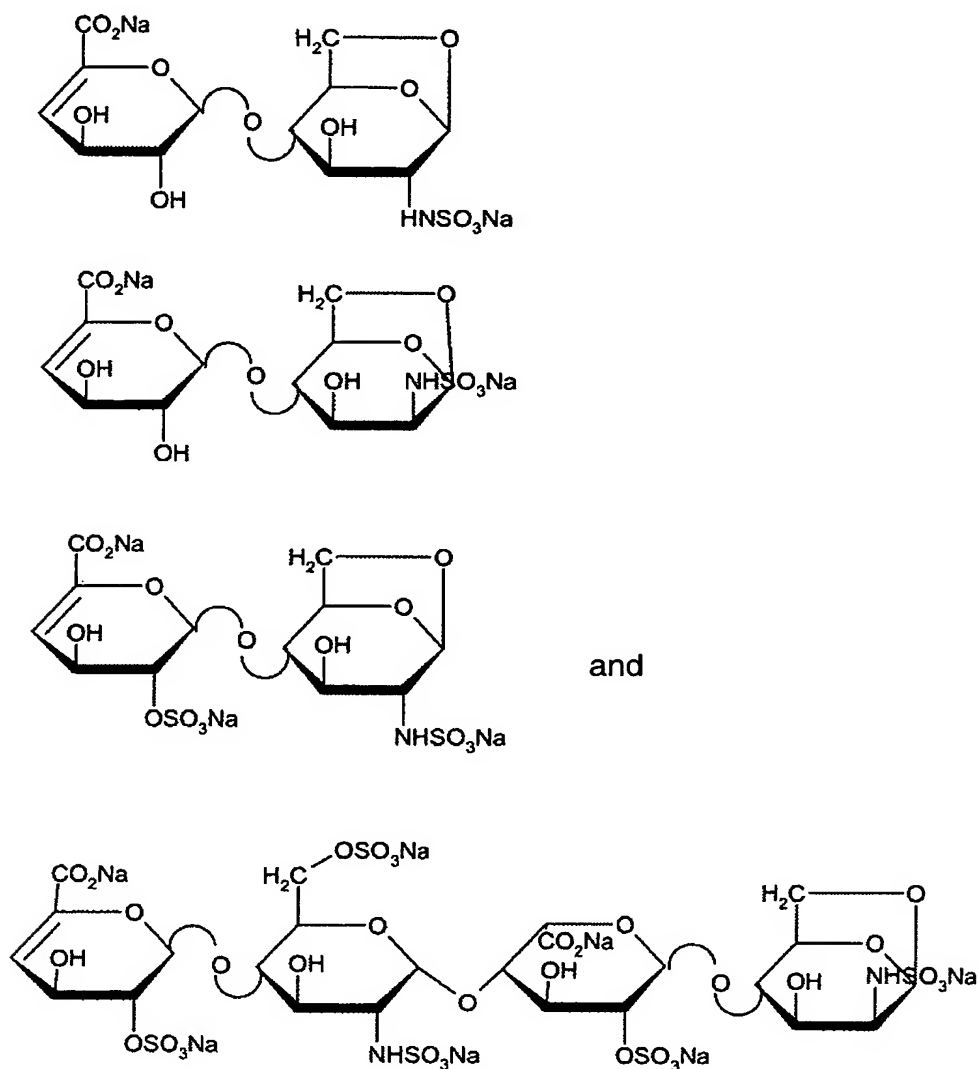
wherein the oligosaccharide chain is in its reduced form.

41. The method as claimed in claim 24 wherein the depolymerized sample comprises at least one oligosaccharide chain whose end is modified with a 1,6-anhydro bond.

42. The method as claimed in claim 41, wherein the at least one oligosaccharide chain is chosen from any of the following:



43. The method as claimed in claim 29, wherein the depolymerized sample comprises a mixture of 1,6-anhydro residues comprising:



44. The method as claimed in claim 43, wherein the mixture of 1,6-anhydro residues range from 15% to 25% of the weight average molecular weight of the sample.

45. The method as claimed in claim 24, wherein the components detected in the depolymerized sample of step (b) are acetylated sugars.

46. The method as claimed in claim 45, wherein the sugars are selectively detected by subtracting an absorbance measured at a wavelength at which both

acetylated and nonacetylated sugars absorb from an absorbance measured at a wavelength at which acetylated but not nonacetylated sugar absorbs.

47. The method as claimed in claim 45, wherein the acetylated sugars detected are selected from acetylated oligosaccharides  $\Delta IVa$ ,  $\Delta IIa$ ,  $\Delta IIIa$ ,  $\Delta Ia$ ,  $\Delta IIa-IVs_{glu}$ ,  $\Delta IIa-IIs_{glu}$ .

48. A method for quantifying the amount of 1,6-anhydro residues in a sample of enoxaparin sodium, comprising:

(a) depolymerizing said sample using a mixture of heparinase 1 (EC 4.2.2.7.), heparinase 2 (heparin lyase II), and heparinase 3 (EC 4.2.2.8.);

(b) reducing the depolymerized sample of step (a); and

(b) detecting the quantity of the 1,6-anhydro residues in the reduced sample of step (b) by high-performance liquid chromatography.

49. A method as claimed in claim 48, wherein the quantity of the 1,6-anhydro residues range from 15% to 25% of the weight average molecular weight of the sample.